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Editorial

PARASITES

In this issue of The Veterinary Journal there is produced an important article on "white spot liver" in the pig. While some evidence has been brought forward by observers in other countries to show that the lesions are associated with parasitic infestation, the results of the work recorded in this article show now that, as far as this country is concerned, there is every reason to believe that the condition is associated with the migration of ascaris larvæ. In addition, evidence is given that other parasites, immature flukes and the intermediate stage of a tapeworm may cause lesions differing somewhat from "white spot liver," but fairly commonly found in the livers of pigs in abattoirs.

These findings are of considerable importance, for apart from the condemnation of livers due to the presence of the lesions, their finding indicates the occurrence of parasitic infestations in the pigs concerned, with consequent effect on the general health of the host.

The part played by the various kinds of parasites in animal economy has been the subject of many contributions to our literature. Under certain conditions parasitic infestations in all classes of livestock are of much importance and their control forms one of the major considerations of animal husbandry. Methods of control are often based on a knowledge of the life history of the parasites concerned and concerning many species we have

detailed information. Research workers in this and other countries have made notable additions to our understanding of the position, with the result that to-day we are in the fortunate position of being able to apply methods of control with much chance of success. The research work involved is tedious and those who devote their lives and energy to such work may not always receive the credit and recognition they deserve. The study of the use of drugs on the animal host involves a knowledge of many of the fundamental principles of science, and in order to establish the true effects of the specified treatment and to know definitely the conditions under which a special drug or group of drugs acts, research workers in different aspects of science are involved. Within recent times the introduction of the drug *phenothiazine* for the control of parasites of the alimentary tract has been followed by reports of good results, but only when certain species of parasites are concerned. Explanations of its selective action still await detailed investigation.

It is encouraging to know that greater support is being given to this important type of research work.

WHITE SCOUR IN CALVES

Diseases of livestock in this country have been shown to be the cause of considerable economic loss. From time to time scientific literature has contained articles relating to the incidence and cause of mortality in calves. White scour, well known to veterinary surgeons and breeders as a cause of heavy loss in some herds, has received due attention from research workers; we venture to state, however, that its cause may not have received the amount of attention it deserves. It is true that all outbreaks do not appear to be due to the same causal agent. Further research is necessary to establish more accurately the various causes and the methods of prophylaxis. In this issue of The Veterinary Journal appears an article dealing with the full investigation of an outbreak in a herd in which losses had occurred over a number of years. It is shown conclusively that the outbreak was associated with *Bacterium enteriditis var. dublin* and that though the disease appeared in the majority of cases to give rise to the typical symptoms and lesions of white scour, in one case, the organism was recovered from a diseased joint. Another interesting observation is that no evidence of carriers was found among the cows; all the observations pointed to a persistent infection in the pens in which the young animals were housed. As our general knowledge of bacteriology and methods of immunisation alter and undergo improvement, there is little doubt that they will be applied to the cause and control of some of our less perfectly understood diseases of livestock, including white scour in calves.

General Articles

INFECTION OF CALVES WITH BACTERIUM ENTERITIDIS VAR. DUBLIN

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It has long been known that in certain localities in Europe, particularly in Denmark, Holland and Germany, serious losses have occurred year after year from what has proved to be salmonella infection in calves. The condition has been described as a form of calf dysentery, white scour in calves or calf paratyphoid. It is not assumed that all outbreaks of so-called white scour are caused by salmonella, but it has been shown that quite a proportion of them has been so caused. The causal agents were first described as " pseudo-typhoid bacilli " by Thomassen in 1897 in Holland, and afterwards as pseudocoli bacilli by Poels in 1899, while later, in 1903 and 1913, they were called paracoli bacilli by Jensen in Denmark, who distinguished them as paratyphi B and Gærtner's bacilli. This was confirmed by Schmitt in 1908, and cases of the kind were described by Luxwolda (1913), Warnecke (1914) and Douma (1916) in Holland. In 1916 in America an outbreak of infectious diarrhœa in calves was attributed by Meyer, Traum and Roadhouse to *Bact. enteritidis*. Strozzi (1934) in Italy found the *Bact. enteritidis* in outbreaks of calf paratyphoid. In East Africa, Daubney proved that outbreaks of calf disease there were due to organisms of the *Bact. enteritidis* type, and in India, Shirlaw (1925) also found a member of the same type in fatal outbreaks among calves. Smith and Scott (1930) identified six strains of the organism from Denmark as *Bact. enteritidis var. dublin*. In 1926 and 1929 Viljoen and Martinaglia investigated calf paratyphoid in South Africa and incriminated *Bact. enteritidis*. Henning in 1939 examined 104 strains of organisms from South African calf paratyphoid and found that two were *Bact. typhi murium*, three *Bact. enteritidis* and ninety-nine *Bact. enteritidis var. dublin*.

In this country, although calf diarrhœa or white scour in calves is not uncommon, only a few outbreaks have been determined to be due to salmonella. In that respect the most convincing evidence has been provided by Bosworth and Lovell in 1931, who described three outbreaks of " dublin " infection in calves in Great Britain. It may well be that the numbers are much greater than at present believed, and that our want of knowledge is based on defective investigation and opportunities.

Two of the outbreaks described by Bosworth and Lovell occurred among two batches of calves about two weeks old, which had been purchased from a dealer for experimental purposes, and the symptoms and losses were observed a short time after they had been delivered. It was supposed that the vitality of the calves had been lowered by exposure during transport, presumably while they carried the infection.

In the third outbreak the disease appeared on a farm in three weeks old calves which had been purchased in the open market a few days previously, and afterwards spread to a number of home-bred calves three to twelve months old. But in none of the outbreaks was the source of the infection ascertained. Presumably the calves had contracted the infection prior to purchase. In any case, it is well known that losses from different causes are often experienced in calves purchased in this way. At the same time, it must be assumed that where a contagious disease such as this appears, it must have a starting place on a farm or dealer's premises.

In an outbreak that recently came under our observation the disease was indigenous on the farm. The calves were not purchased, but born and reared on the premises. In that, and a few other respects, the outbreak which we have investigated and now put on record differed from those described by Bosworth and Lovell.

History of Outbreak.—In a dairy farm in Cheshire it was reported in July, 1940, that losses had been occurring among the calves for some years, and that on an average 20 per cent. were lost every year. On the farm of about 130 acres there were upwards of 100 head of stock, all British Friesians. The owner has another farm under different management about half a mile away on which no losses have been experienced.

On the farm where losses are occurring the calves are all born and reared on the place. They are fed twice daily at first on the colostrum, and for a short period afterwards on the milk of the dams, but later on mixed milk. No trouble arises if the calves are born and suckled on their dams at pasture.

The affection appears only when the calves are housed. They are housed in loose boxes and several are often put together in the same box after they calve. These boxes are stated to be thoroughly disinfected when they are empty and before further use. The litter used is short straw, but it does not appear to be frequently changed, and where several calves are kept it was noted that it had been grossly contaminated with the droppings of the calves. Recently it had become the practice to dress the umbilicus of the calves after birth with iodine preparations.

Symptoms.—Symptoms are usually shown during the first few weeks of life, but are sometimes deferred until the animals are four to eight weeks old.

When the farm was first visited on July 24, 1940, four calves, consisting of two bulls and two heifers, were ill out of about 15. They were all about

six weeks of age. The sick calves had been isolated in pairs in two separate boxes. One of them, a heifer calf, had become ill on July 22. It appeared very depressed, head and ears drooping and the coat very dull and staring. Its eyes were retracted and its appetite moderate. It had some diarrhœa and it was rapidly failing in condition. Its temperature on the 23rd was 105 degrees F., and on the 24th, 103.5 degrees F. Its respirations were rapid and blowing, and it had an occasional cough. The umbilicus was thick, but healed. The other calf with it was only showing slight symptoms of the same nature. The calves had received a dose of liquid paraffin, and were also being treated with sulphanilamide. The seriously affected calf died on July 29, and no post-mortem examination was made. The other calf recovered, but remained unthrifty for a time.

The two affected bull calves had remained normal until one month old, when the symptoms developed. They were then receiving half a gallon of milk twice daily. Both were affected with liquid yellow or greenish fœtid diarrhœa. In both cases the coat was dry and staring, the ears depressed, and the abdomen tucked up and flanks hollow. The umbilicus was healed, but the cord was thick. The temperature was 101 degrees F. in one, and 102 degrees F. in the other. These calves remained alive and unthrifty until they were forwarded for examination, and are referred to later as Nos. 3 and 4.

Among older calves which survived, it was noted that they did not thrive so well or show such bloom as the calves on the other farm, and their coats remained ragged for a time.

It was arranged that a number of affected calves which were not likely to live should be forwarded to the Department for investigation.

Examination of Affected Calves. Calf 1.—A heifer calf, seven weeks old. It was normal on the morning of July 25, and comatose in the afternoon, when it died, and was dispatched for examination.

Lesions.—Tissues pale. Some fine granulation-like patches on the peritoneum over rumen and omentum; small petechiæ over the grooves of the heart and one hæmorrhage on the bicuspid valve; some petechiæ in the cortex of the kidneys; irregularly congested areas in liver; mucous membrane of the abomasum along the folds œdematous and reddened; patchy congestion in the anterior portion of the small intestines and slight congestion in the large intestines, the contents of the latter being fluid; reddening of the synovial membranes and considerable quantities of clear fluid synovia in the main joints; a small abscess with grey-green purulent contents inside the umbilicus. In films from the umbilical abscess there were numerous gram-negative short bacilli and some diphtheroids.

In cultures on serum agar, mixed colonies of coliform organisms and diphtheroids were obtained from the umbilical abscess and the joints, but from the heart, liver and kidneys pure cultures of coliform organisms were obtained.

XUM

These latter organisms were actively motile in four to eight-hour broth culture, produced no indol in peptone water, and rendered litmus milk alkaline. They were H_2S positive and produced acid and gas in mannite, glucose and maltose. No change occurred in lactose and saccharose. It was evident, therefore, that they belonged to the salmonella group, and as they gave a higher agglutination titre with *Bact. pullorum* serum than a strain of *Bact. pullorum*, they probably belonged to the same group as that organism. Their further examination will be referred to later, with the organisms obtained from other cases.

Calf 2.—Born on July 24 and was apparently normal on the morning of August 1. In the afternoon it had a slight nasal discharge and was very intoxicated. It was sent for examination on August 2. When received its condition was found to be very poor, the coat dry and staring, and its flanks hollow; the animal was very weak and inclined to remain down most of the time. There was a whitish discharge from the nostrils. There was slight salivation, with the tongue slightly protruded. Its eyes were full and prominent. Its tail and quarters were soiled with greenish-white, semi-solid fæces. The calf drank water eagerly. The stump of the umbilical cord was dry and black, but not detached.

On August 3 its temperature was 104.5 degrees F., and it was very dejected. It was then destroyed.

Lesions.—Carcase œdematous and very poor. A small quantity of clear serous fluid in peritoneal and pleural sacs; numerous small petechiæ on the mucous membrane of the pharynx, epiglottis and larynx; small areas of atelectasis in the apical, cardiac and lower portions of the main lobes of the lungs, heart soft and pale and a few petechiæ on the coronary groove; spleen very large; a few soft brown calculi in the gall-bladder, the mucous membrane of which carried a few petechiæ; numerous small hæmorrhages on the peritoneum over the stomach and intestines, mesentery and omentum; slight œdema of the folds of the mucous membrane of the abomasum; patches of congestion on the mucous membrane of the intestines, including the ileocæcal valve; intestines nearly empty; nearly all joint capsules hæmorrhagic, and a few hæmorrhages on cartilages, while synovial fluid was in excess; umbilical cord contained a thrombus in the vessels but no abscess.

MacConkey's and plain agar plates were inoculated from the heart-blood, spleen and liver and, in 24 hours, a pure culture of organisms identical with those in the first case was obtained. Suspensions of the organism were agglutinated by *Bact. pullorum* serum in high dilutions. A pure growth of the same organism was obtained on MacConkey's medium from tetrathionate broth inoculated from the intestinal contents. Only staphylococci, probably contaminants, were isolated from the umbilicus.

Calf 3.—The calf was approximately eight weeks old, and was one of the

two bull calves which had been seen a month previously. It was very unthrifty and still suffered from diarrhœa. The right stifle joint was enlarged and the animal was lame on the right hind limb.

This animal was received 7/8/40, and destroyed on 8/8/40. The temperature of the animal during this interval was 102.4 degrees F. and 103 degrees F.

Lesions.—Tissues pallid, heart-wall flabby and one cusp of bicuspid valve studded with hæmorrhages; the folds of mucous membrane of the abomasum slightly thickened; reddening and slight thickening of patches of mucous membrane of the small intestines in posterior part and over Peyer's patches and in a few areas in the large intestines; right stifle joint enlarged and contained a great deal of fibrin and opalescent fluid. In this and other joints some hæmorrhages in the tissues around the joints as well as some fluid and fibrin.

Culture media were inoculated from heart-blood, liver, spleen, joints and umbilicus. Salmonella organisms grew only from the right stifle joint. These organisms were identical with those in the previous case. No salmonella were found in the fæces of this calf.

Calf 4 was the fellow of calf 3, which was seen a month previously. It was approximately eight weeks old and had been affected for about a month. The symptoms were similar to those shown by calf 3, except that none of the joints appeared to be affected and it was not lame. The temperature, however, was higher, the records being as follows:

7/8/40	... 104.5° F.	...	10/8/40	... 103.4° F.
8/8/40	... 105.5° F.	...	11/8/40	... 103.4° F.
9/8/40	... 105.2° F.	...	12/8/40	... 101.2° F.
				(animal moribund)

The animal was destroyed on 12/8/40.

Lesions.—Condition very poor; broncho-pneumonia in patches on cardiac, apical and main lobes of both lungs, with one little abscess in an apical lobe and a few small lobular hæmorrhagic patches; heart-wall flabby; pinkish red liver; abomasal folds œdematous and thickened and presented a few petechiæ, abomasal contents mucoid; mucous membrane of anterior portion of small intestines thickened, wrinkled and bile-stained, and in the latter portion of small and large bowel patches of congestion and hæmorrhages, contents mucoid and mixed with a little blood; hock and elbow joints contained an excess of synovia and small flakes of fibrin, synovial membranes swollen and pink, and a few hæmorrhages on surface of bone. In the abscess in the lungs cocci only were present both in stained films and cultures.

Culture media inoculated from the heart-blood, bile from the gall-bladder, intestinal contents and mesenteric glands yielded growths of salmonella similar to those of previous cases.

Identification of the Organisms.—All the organisms isolated were compared

on different media and found to be identical. As already pointed out for the first case, the organisms formed colourless colonies on MacConkey's medium, were non-lactose fermenters, rendered litmus milk alkaline, did not produce indol in peptone water, were H_2S positive, and were actively motile and presented the usual other characteristics of the salmonella group.

Carbohydrate Fermentations.—Cultures of the strains from the various calves were grown in peptone water containing 0.5 per cent. of requisite carbohydrate and Andrade's indicator. They were cultivated at 37 degrees C. for 18 days, and any changes noted at daily intervals. The changes noted were identical for each calf, and are given in the following table:

Lactose.	Glucose.	Maltose.	Mannite.	Saccharose.	Salicin.	Xylose.	Inosite.	Rhamnose.	Arabinose	Dulcite.
—	AG1	AG1	AG1	—	—	AG2	—	AG1	AG9 to 10	AG1

AG = formation of acid and gas, and the following numeral denotes the time in days taken for the reaction to appear. — = no change.

Serological Tests

"O" agglutinins.—Since the enzymic reactions of the organism from the calves pointed to the identity of it as *Bact. enteritidis var. dublin*, a preliminary agglutination test was carried out with pullorum serum.

Bact. pullorum being a non-motile organism, it only contains "O" agglutinogen, and the serum produced by this organism contains only "O" agglutinin. Moreover, the "O" antigen IX points to the group species which includes *Bact. typhosum*, *Bact. enteritidis* and its varieties, *Bact. gallinarum* and *Bact. pullorum*. "O" suspensions were made from the calf organism and from a culture of *Bact. enteritidis v. dublin*, and compared with the reaction of *Bact. pullorum* to the serum. The following are the results, and they were identical for the cultures from all calves:

Dilutions of serum	40	80	160	320	640	1280	2560
Calf Strains	+++	+++	+++	+++	++	+	—
Bact. enteritidis var. dublin	+++	+++	++	++	+	+	—
Bact. pullorum	+++	+++	+++	+++	++	+	—

+++ = complete agglutination. + = slight agglutination.

It was thus evident that the organisms from the calves belonged to salmonella group D, and since they were motile they were probably varieties of *Bact. enteritidis.*

For the "O" suspensions smooth colonies were grown on agar slopes for 24 hours at 37 degrees C. The growth was then washed off with 95 per cent.

alcohol and heated in a water bath at 56 degrees C. for 2 hours. The suspension was then centrifuged and the alcohol pipetted off. The deposit was afterwards suspended in one cubic centimetre of distilled water and made up with normal saline to the required opacity (Henning). All "O" agglutination tests were read after 24 hours in the water bath at 55 degrees C.

"H" agglutinins—"H" suspensions of strains of the calf organisms and of *Bact. enteritidis v. dublin* were prepared by cultivating the organisms in broth for six hours at 37 degrees C., killing the bacteria by the addition of 0.25 per cent. formalin, and heating for two hours at 55 degrees C.

These suspensions were tested against dilutions in normal saline of a known "Dublin" serum stated to have an "H" titre of 1 in 16,000. The mixtures were incubated for two hours at 55 degrees C. The floccular agglutination was comparable in both cases. It was complete in both cases up to dilutions of 1 : 20,000, but was shown partially in dilutions up to 1 : 40,000, and was more marked for the calf strain than the "Dublin" strain.

Absorption Agglutination Tests

For the purpose of absorption, cultures of calf and "Dublin" strains were incubated for 18 hours at 37 degrees C. and the same serum used as for "H" agglutination tests. In the attempts at complete absorption it was found that the procedure commonly advised was insufficient for both organisms. It may be that the primary dilution of serum of 1-25 was too low. The procedure adopted consisted in mixing 1 c.c. of 1-25 Dublin serum with 1 c.c. of thick suspension in saline of the relative organism, incubating the mixture at 37 degrees C. for 24 hours, and then centrifuging for 15 minutes at 3,000 r.p.m. The supernatant fluid was then pipetted off, and to it was added another 1 c.c. of thick suspension of culture and incubated for another 24 hours at 37 degrees C. This was centrifuged again and the supernatant fluid was then taken off and made up with normal saline to give a dilution of serum of 1-100.

It was then tested against "H" suspensions of the two organisms. The result was complete absorption of the H. agglutinin as indicated in the following table:

Absorption of serum prepared against a strain of "Dublin."

Bacterial Suspension	Serum absorbed with						Unabsorbed serum controls 20,000
	Calf suspension			Dublin suspension			
	dilutions			dilutions			
	200	400	800	200	400	800	
Calf "H" ...	—	—	—	—	—	—	+++
Dublin "H" ...	—	—	—	—	—	—	+++

A complete "Mirror" test was carried out with calf strain and a positive serum was obtained by inoculating a rabbit with a series of doses of a suspension of an 18-hour agar culture to which was added 0.25 per cent. formalin, and which was afterwards heated in a water bath for two hours at 57 degrees C.

The serum from the rabbit had an "H" titre of 1 : 40,000 and "O" titre of 1 : 640 against the calf strain, and a strain of "Dublin" obtained from Dr. W. M. Scott.

The saturation of serum with calf strain and Scott's Dublin strain "H" suspension was made in three stages, so that the final dilution of saturated serum preliminary to test was 1-100, as for the previous absorption tests, with the following results:

"H" agglutinin.

Absorption of serum prepared against Calf Strain.

Bacterial "H" suspension	Serum absorbed with Calf strain dilutions 200	400	800	Scott strain dilutions 200	400	800	Positive control 4,000
Calf strain ...	—	—	—	—	—	—	+++
Scott Dublin strain	—	—	—	—	—	—	+++

"O" agglutinin.

Absorption of serum prepared against Calf Strain.

Bacterial "O" suspension	Serum absorbed with Calf strain 200	400	800	Scott strain 200	400	800	Positive Control 620
Calf strain ...	—	—	—	—	—	—	+++
Scott Dublin strain	—	—	—	—	—	—	+++

Discussion.—From the examination of the calves it would appear that in one case infection had entered the umbilicus, but in the other three absence of infection in this situation suggested the entrance of infection by some other route, and it was probable that that route was the alimentary canal. At any rate, the presence of the organisms in the alimentary ingesta of two of the animals pointed to the fæces as a potent agent for the transmission of the disease. Calf 4 had been showing symptoms for one month, and probably had been discharging the infection for that length of time. How long a calf may continue after recovery to remain a carrier is not determined. In any case, what evidence there is suggests that carriers are not common, and recovered

animals soon free themselves of infection (Henning). But the viability of the organisms in the fæces appears to be long, and if the litter is not frequently removed from the boxes in which the calves are housed, massive infection is more liable to take place. According to Henning (1939), the organisms in dry manure remained viable for 1069 days when the material was kept in a bottle on a shelf in the laboratory.

How the disease was first introduced is a matter for speculation. It is, however, probable that it has been kept going by the succession of calvings and the infected premises. There is, of course, the possibility that some of the cows may be carriers, but on the other hand the experience has been that if a cow is allowed to calve and suckle its calf in the field the calf remains free from infection, suggesting that the milk and the droppings of the cow are not a source of serious infection in the calf, and need not be taken into account in the control of the disease.

In most recorded outbreaks of paratyphoid disease in calves, the adult cattle have shown no symptoms, and in the outbreaks noted in the latter there has been no history of trouble among the calves. In this particular outbreak in calves the cows showed no evidence of infection. Cases of salmonella infection in both cows and calves were noted by Bourner and Doetsch (1928) and Pröscholdt (1931).

In order to determine whether any members of the herd were carriers of the infection, samples of blood and fæces were collected from the 36 cattle in the milking herd and 26 samples of milk from the cows in milk. The samples of milk and of fæces were tested by seeding in the enrichment medium tetrathionate broth, and afterwards sowing from this on to MacConkey's agar plates and incubating for 24 hours. In some cases Wilson and Blair's medium was used as well as MacConkey's medium. In all cases the results were negative and no colonies of salmonella were isolated. We have found that the Wilson and Blair's medium is an excellent one for distinguishing the salmonella because the colonies are black and the medium around them is also stained black, and has a metallic sheen, but on trial we did not find that in this medium appreciably more salmonella grew from a known dilution than in MacConkey's medium.

The sera from the blood samples were submitted to the agglutination test for "H" and "O" agglutinins of the strains of *Bacterium enteritidis var. dublin* isolated from the calves. The results were as follows in regard to the agglutination titres:

	No. of samples with varying titres.						
	Nil.	1 : 20	1 : 40	1 : 80	1 : 160	1 : 320	1 : 640
"H" agglutinin ...	7	7	5	9	5	3	—
"O" agglutinin ...	2	3	11	16	4	—	—

At the end-point agglutination was not usually complete, and in some of the samples the fluid was not water-clear even in the lower dilutions. As a rule, when the titres were low for "H" agglutinins, they were also low for "O" agglutinins. Altogether the whole of the evidence pointed to the absence of carriers among the dairy cows.

It has therefore been recommended in this outbreak:

1. That no more calves for the present be housed in the boxes now being used, and that those now being reared be removed.

2. That when the boxes are vacant, all the litter and droppings in the boxes, in the yard and manure heap be disinfected and removed to arable land as soon as possible, and that the boxes be thoroughly scrubbed out and disinfected.

3. That each calf be housed in a separate pen which has been thoroughly disinfected before use.

4. That the umbilical cord and umbilicus be treated with iodine preparations at birth.

The value of these precautions has yet to be ascertained. Vaccines or sera have not so far been considered as valuable preventive agents, nor have the sulphanilamide compounds been shown to have any appreciable beneficial effect in the curative treatment of the affection.

Summary

1. An outbreak of calf disease associated with *Bacterium enteritidis var. dublin* is described.

2. The disease has been appearing for a number of years in a herd.

3. The organism was isolated from various organs and from the fæces and bile, and in one case from a diseased joint.

4. No evidence was found of carriers among the cows.

5. Recommendations are made for the eradication of the disease by sanitary measures.

We desire to acknowledge our grateful thanks to Dr. W. M. Scott for a culture and a sample of *Bact. enteritidis var. dublin* serum for serological tests, and for confirming our observations regarding the identity of the organism. We also wish to thank Mr. W. Kendrick, M.R.C.V.S., Tarvin, for securing the material for examination.

REFERENCES

Arkwright, J. A. (1931): *A system of Bact. M.R.C.*, *6*, 381.
Bosworth, T. J., and Lovell, R. (1931): *Sec. Rep. Inst. Animal Path.*, University of Cambridge, 21.
Bourmer and Doetsch (1928): *Z. Fl. u. Milchhyg.*, *38*, 389.
Daubney, R. (1927): *Vet. Rec.*, *7*, 793.

Douma, S. (1916): *Tydsch. v. Diergen.*, *43*, 720.
Henning, M. W. (1939): *Onderstepoort J. Vet. Science & Animal Industry*, *13*, 79 (with extensive bibliography).
Jensen, C. O. (1913): *Handbach der Pathogenen Mikri-organismen.*, 6, 121.
Martinaglia, G. (1929): 15th *Rep. D.V.S.*, South Africa, 233.
Meyer, K. F., Traum, J., and Roadhouse, C. L. (1916): *J.A.V.M.A.*, *2*, 17.
Pröscholdt, O. (1931): *Z.f. Infkr.* (Haust), *39*, 115.
Schermer u. Ehrlich (1921): *B.T.W.*, *37*, 469.
Schmitt, F. M. (1908): *D.T.W.*, *16*, No. 47, 673.
Shirlaw, J. F. (1925): *Ind. Jl. of Vet. Sc.*, *5*, 232.
Smith, J., and Scott, W. M. (1930): *Jl. Hyg.*, *33*, No. 1, 32.
Strozzi, P. (1934): *Clin. Vet. Milano*, *57*, 337.
Thomassen, M. (1899): *Ann. Inst. Pasteur*, *11*, 523.
Viljoen, P. R., and Martinaglia, G. (1928): 13-14 *Rep. Div. Vet. Educ. Res.*, 535.
Warnecke, H. (1914): *Tydsch. v. Veeart*, *41*, 357.
White, F. Bruce (1930): *Jl. Hyg.*, *29*, 443.

CHRONIC FOCAL INTERSTITIAL HEPATITIS IN THE PIG

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Introduction

THE condition or group of conditions dealt with in this paper consists of localised areas of chronic inflammation, mainly superficial, which are commonly found in the liver of pigs. The most common type is seen as a varying number—from one or two up to 50 or more—of whitish foci with an indefinite boundary extending in the form of thickened interlobular septa which gradually merge into the apparently normal septa clearly visible in the liver of pigs more than a few weeks old. (Figs. 1-4.) The lesions are seen in histological section to extend further than is apparent macroscopically and interlobular septa a considerable distance from the macroscopic lesion may be affected. In recent foci the centre is usually composed of one or more lobules which are hæmorrhagic, whereas in older lesions it often takes the form of an opaque whitish nodule. Some foci, however, have no opaque centre but consist merely of thickened septa without obliteration of the structure of the central lobules. The lesions are usually most numerous on the surface and extend for only 2-3 mm. beneath the liver capsule, but similar foci are also found in the substance of the organ. This condition is often termed "white spot liver" or, by some inspectors, "milk spot." The latter term is unsuitable as it does not describe the lesion and refers to quite different changes which take the form of areas of opacity of the liver capsule without involvement of the underlying liver parenchyma and with no gross structural changes.

A second type of lesion sometimes seen consists of "pearly" nodules up to 5 mm. in diameter, situated just beneath the liver capsule.

An entirely different lesion appears as spherical nodules up to the size of a pea, or even larger, containing a brownish material within a thick fibrous capsule. Such lesions are usually considered to be parasitic by meat inspectors. These lesions are circumscribed and do not always show an extension in the form of thickening of the surrounding interlobular septa.

Incidence of the Condition in Great Britain and Ireland

In order to obtain information as to the incidence of lesions of the above types among pigs in the British Isles and Eire and the opinion of meat inspectors as to their nature, a questionnaire was sent to 40 veterinarians and sanitary inspectors engaged in meat inspection at abattoirs and bacon factories. A total of 14 replies was received, but it is fortunate that these came from a very wide area, namely, the counties of Derbyshire, Devon, Essex, Glamorgan, Gloucestershire, Hertfordshire, Lancashire, Staffordshire, Suffolk and Worcestershire, as well as from Eire (Dublin) and Northern Ireland (Belfast). A brief summary of the information supplied is given.

Number of pigs slaughtered and incidence of "white spot liver."—Twelve inspectors gave figures for the number of pigs slaughtered annually. The total is 1,643,000, the number at individual centres varying from 5,000 to 900,000. The incidence of "white spot liver" is given as varying in different centres from one per cent. to 50 per cent. of all pigs slaughtered. The average incidence, taking into account the number of animals slaughtered annually at different centres, is 10 per cent. These figures include the three types of lesion described above, but there is complete agreement that the first type is by far the most common, forming 70-98 per cent. of all the cases observed.

Variation in different centres.—There is insufficient evidence to warrant any conclusive statement on this point, since in most centres no accurate statistics are kept. One inspector stated that the incidence was almost twice as great in English pigs (6 per cent.) as in Irish pigs (3.5 per cent.) killed at the same centre. The highest incidence was recorded in Glamorganshire (50 per cent.) and Worcestershire (40 per cent.), both figures referring specifically to porkers. The lowest incidence was recorded in Essex (1.6 per cent. and 2 per cent.).

Age of pigs affected.—All except two inspectors were agreed that the incidence is higher in porkers and store pigs than in sows and boars. The figures of 40 per cent. and 50 per cent. given refer to porkers, and it was suggested that the lesions are about twenty times as common in porkers and store pigs as in bacon pigs.

Source of pigs and seasonal incidence.—Four inspectors considered that the incidence varied with the source; the others were unable to give any information on this point. One inspector recorded 33 cases of "white spot liver" among 49 unweaned stores from a group of 60 casualty pigs. The re-

mainder comprised two boars and nine sows, all of these being free from lesions. The incidence in the young pigs was thus over 66 per cent. Six inspectors considered that there was no seasonal variation in the incidence, two believed it to be more common during the winter months, and the remaining two who replied to this question stated that they had no information either way.

Type of lesion and distribution.—The first type of lesion was said to be the commonest encountered, the last of the three types being usually classed as "parasitic," as are the greyish "tracks" sometimes seen beneath the liver surface. It was generally agreed that the lesions are mainly superficial, but also occur in the depth of the liver. Six inspectors stated that they are most common on the anterior (diaphragmatic) surface of the liver, four considered them more common on the posterior surface, and one thought they were equally distributed over both surfaces.

Ætiology.—The views expressed varied widely. For the first type of lesion three inspectors suggested parasites as the cause, whilst six blamed dietary factors, especially present-day "balanced rations."

The results of the inquiry are of particular interest as we are not aware of any data relating to this condition as it occurs in the British Isles. The figures quoted are, however, only a rough guide, since the condition is not one of which records are commonly kept. In any case, the keeping of statistics in abattoirs and bacon factories is very variable, ranging from complete absence to a fairly detailed recording system. The replies to the questionnaire show that the condition is very common and probably occurs in about 10 per cent. of all pigs, being more common in porkers and store pigs than in older animals. There is no agreement as to its ætiology. Affected livers are dealt with according to the severity of the condition, a few lesions when present being merely excised, whilst badly affected livers are condemned. It is clear that the loss of valuable food material is considerable, but this is not of necessity the most important aspect of the problem. Whatever the ætiology, the liver damage which precedes and accompanies the lesions probably affects the animal's health, and if the condition should be parasitic it is likely that the parasite would produce lesions in other organs or tissues.

Review of Literature

The older textbooks dealing with meat inspection do not mention "white spot liver," but merely refer generally to cirrhosis. Ostertag's textbook (1934) gives an excellent illustration of the lesions (p. 149, Fig. 84), and records that in earlier editions the author expressed the opinion that they are due to ascaris larvæ. It is stated that the condition is found in twenty to twenty-five per cent. of pigs. Mönnig (1934) states that ascaris larvæ during their migratory period may cause destruction of liver tissue with

hæmorrhage, especially around the intralobular veins, although the most important lesions are found in the lungs. Udall (1939) gives a good account of ascaris infestation in pigs, in which he refers to the liver damage produced by the migrating larvæ. The pathological changes are said to be an initial congestion and hæmorrhage, followed by the formation of whitish indurated areas about ¼ inch in diameter. A similar description is given by Kaupp (1925). A number of other texts have been consulted, but no reference was found in them to liver damage produced by migrating ascaris larvæ.

The earliest reference we have found to a condition apparently identical with "white spot liver" is at the end of a paper by Junack (1907) on Tuberculosis in the Pig, in which he describes lesions somewhat similar to those occurring in the liver of pigs following infection with avian tubercle bacilli. They were found in 1-2 per cent. of pig livers examined in Breslau abattoir, and consisted of focal areas of cirrhosis, the larger ones having a solid greyish centre often containing a small hæmorrhagic area. The lesions extended for some distance along the interlobular septa, and sections showed a massive infiltration with eosinophil leucocytes. In addition, there were pea-sized spherical lesions with a necrotic caseous centre, from one of which a parasite was isolated. This was considered to be *Œsophagostomum dentatum* but, when Bugge and Müller (1928), working some years later in Junack's laboratory, isolated young *Fasciola hepatica* from pig livers, Junack suggested that the parasite formerly isolated was probably a young Fasciola.

Joest and Felber (1908) described 16 cases of a condition in pigs which they called "chronic multiple interstitial hepatitis." The number of lesions present varied from 8-10 up to such large numbers that little normal liver tissue was visible. A detailed description of the lesions is given, as well as an illustration which was later reproduced in Joest's textbook of Veterinary Pathology (1936), as well as in that of Nieberle and Cohrs (1931). No bacteria were isolated from the lesions, and the parasites suggested as being responsible were larvæ of *Strongylus paradoxus* or Onchospheres of *Tænia hydatigena*, the former being favoured. In five cases of diffuse cirrhosis in the pig examined at the same time there was no eosinophil infiltration.

Local eosinophilia in the pig's liver was dealt with in detail by Schmey (1911), who found lesions similar to "white spot liver" in 25 per cent. of livers examined. He considered the cause to be parasitic, but although he examined "hundreds of lesions" he failed to isolate a parasite. He did not agree with Joest's suggestion that *Strongylus paradoxus* might be the cause, but suggested ascaris, echinorhyncus or flukes. He termed the condition "multiple fibrous eosinophilic hepatitis of unknown ætiology."

The earlier literature dealing with "multiple interstitial hepatitis" was reviewed by Bugge and Müller (1928), who recorded the finding in an 18 months old pig of four young flukes in three lesions. There were 25 lesions present,

Plates I-III illustrate the commonly occurring lesions of "white spot liver." Plate IV shows the larger and less common type of lesion containing immature flukes.

FIG. 1.—Posterior surface of liver of pig 24, showing typical lesions of "white spot liver." x 1/5.

FIG. 2.—Anterior surface of same liver as Fig. 1, showing similar lesions. x 1/4.

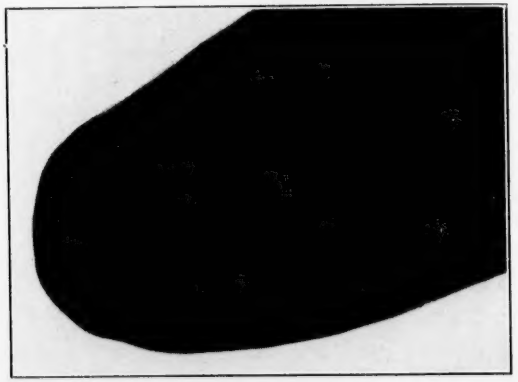

FIG. 3.—Anterior surface of portion of right lateral lobe of above, showing six lesions formed by thickening of interlobular septa. x 2/3.

FIG. 4.—Small portion at junction of left lateral and left central lobes of above, showing four lesions, one having a well defined opaque centre. x 1½.

and from their description they seem to correspond with the third type described at the beginning of this paper. We have found immature flukes in similar lesions in pig livers in this country, but the condition must be distinguished from the commonly occurring " white spot liver." Lesions due to flukes had previously been described by Frenkel (1919) under the name *Distomatosis hepatis suis.* He isolated from a single lesion a young *Fasciola hepatica* 1 mm. in length, and considered that lesions of this type result from the hæmatogenous transfer of cercariæ from the gut to the liver and their failure to reach the bile ducts, followed by encapsulation and disintegration at an immature stage of development. The lesions may subsequently calcify and the condition is considered of no importance in the spread of the parasite because of its failure to become mature.

The isolation of a calcified nematode larva from a " pearly cyst " on the surface of a pig's liver was described at a meeting of the Helminthological Society of Washington in 1929 by Schwartz and Alicata. The affected liver showed considerable mottling due to increase in the thickness of interlobular septa. Areas of fibrosis which were rather similar to the above but extended much more deeply into the liver parenchyma had been previously described in the pig's liver by Schwartz and Price (1929), following migration of *Stephanurus dentatus* (swine kidney worm). This finding of " pearly cysts " containing degenerated nematode larvæ was again recorded by Schwartz and Alicata in 1933, when they stated that the parasites " can be considered ascaris with a fair degree of certainty." The lungs of affected pigs showed ascaris larvæ which were larger in size than those in the liver, and this is in accord with the known facts of the migration and development of the pig ascaris. It seems fairly certain that the lesions described in the U.S.A. by these authors are the same as those of " white spot liver " in this country. Hall in 1924 (quoted by Schwartz and Alicata) suggested that " the common parasitic cirrhosis in swine livers may yet be shown to be due, at least in part, to these (ascaris) larvæ." Histological sections of the lesions described by Schwartz and Alicata showed an increase in interlobular connective tissue accompanied by eosinophil infiltration. The possibility of the kidney worm as the causal parasite was excluded because of the different character of the lesions and from the observation that lesions of the " pearly cyst " type were found in pig livers in areas where *Stephanurus* does not exist but where ascaris is present. The epidemiological evidence thus fits in with the known distribution of ascaris in swine, but not with that of the kidney worm.

Nieberle and Cohrs (1931) in their Textbook of Veterinary Pathology fail to distinguish the lesions of ordinary " white spot liver " from the spherical nodules of the type from which Bugge and Müller isolated young flukes. An illustration of the latter type of lesion is given, and the authors state that they have confirmed Bugge's findings. The recent edition of Joest's textbook, re-

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vised by Dobberstein (1936), gives the most complete account available of parasitic cirrhosis in animals. The macroscopic and microscopic description given resembles that of "white spot liver" as it occurs in this country. Eosinophil infiltration is stated to be so intense as almost completely to mask the great increase in periportal connective tissue. In old lesions there are fewer eosinophils, but these cells are always a characteristic feature. The centre of the lesions often consists of a mass of débris, red cells and broken-down blood pigment. The author appears to hold the view that the lesions are the same as those from which Bugge and Müller isolated flukes, but he differentiates them from the borings made by the wandering Onchospheres of *Cysticercus tenuicollis* which also produce liver destruction and provoke a focal eosinophil reaction. The possibility of ascaris larvæ as a cause of "multiple interstitial hepatitis" is mentioned, but Dobberstein states that the parasites have never been demonstrated.

Roberts (1938) in New Zealand has published a monograph on the life history of *Ascaris lumbricoides* which includes a study of experimental infestation in pigs. In some animals he describes lesions which seem identical with those of "white spot liver" in this country, and this is borne out by a photograph which is exactly similar to the naturally occurring condition.

During the preparation of this paper an article by Schwartz was consulted which clearly shows that a condition apparently identical with "white spot liver" in this country is recognised in the United States as being due to ascaris larvæ. This paper is the United States Department of Agriculture Bulletin No. 1789, "Internal Parasites of Swine," and it includes an illustration of the condition with the legend "Swine liver showing numerous small scars (whitish areas) produced by migrating ascaris larvæ" (page 22, Fig. 16). In the text it is stated that the liver is sometimes permanently affected by the massive migration of young worms, and that in some batches of pigs as many as 35 per cent. of the livers may be affected.

No work appears to have been published in this country on "white spot liver," and the balance of opinion in abattoirs seems in favour of a nutritional origin, although the focal nature of the lesions and the intensity of the eosinophil infiltration are clearly in favour of a parasitic lesion.

Personal Observations

A useful basis for the study of pathological conditions in the pig's liver was afforded by preliminary work on the normal liver of this species which has already been published (White, 1939a). The material used in this early work consisted of samples of liver from 50 pigs, mainly young animals, and included five cases of "white spot liver." Some work was also carried out on the effect of carbon tetrachloride on the pig's liver (White, 1939b), and this showed that the cirrhosis following repeated doses of this drug

was not accompanied by eosinophil infiltration. This applied also to the acute degenerative changes produced by large single doses of the drug. It is of interest also that no evidence of ascaris infestation was found at autopsy in any of the 25 pigs used in these experiments, and that there was no case of "white spot liver."

Further material was obtained from a large bacon factory where "white spot liver" is commonly found, and also from various veterinary surgeons and local abattoirs.

In all we have examined histologically one hundred pig livers, including 26 cases affected with "white spot." We have also carried out preliminary experiments in collaboration with Dr. J. N. Oldham in an attempt to reproduce the condition by feeding embryonated ascaris eggs, and the results will be presented in a subsequent paper.

Technique.—In the majority of cases we were unable to examine the whole liver and were restricted to small pieces illustrating typical lesions and already fixed in formol saline. The material was embedded in paraffin and the blocks were examined by cutting and staining sections taken at intervals of about 50 microns in order to search for evidence of parasites in the lesions. Sections were stained as a routine with hæmatoxylin and eosin. Formalin-fixed material is unsuitable for dissection but we were able to obtain a number of lesions in the fresh state, and from one of these a nematode larva was obtained.

Types of lesion.—1. "White spot liver." This is the commonest type of lesion. The number of lesions vary from a few to well over a hundred, and are for the most part situated superficially, although deep lesions are usually found if the whole liver is cut into thin slices. Early lesions show a hæmorrhagic centre composed of one, two or three liver lobules. Older lesions show an opaque whitish centre from which radiate thickened interlobular septa or, alternatively, the whitish centre is absent.

Histological sections of early lesions show hæmorrhage and necrosis of liver tissue which vary in extent, involving sometimes only small groups of liver cells in the centre or intermediate zone of a lobule, whilst in other cases as many as three complete lobules may be destroyed. The area is infiltrated by enormous numbers of eosinophils and a few neutrophils, and this infiltration extends for some distance along the surrounding periportal connective tissue, and is still evident far beyond the macroscopic boundary of the lesion. Fibroblast proliferation rapidly occurs and a considerable amount of new fibrous tissue is laid down in the septa so that old lesions usually show grossly thickened septa with fibroblasts and collagen fibres often almost masked by enormous numbers of eosinophils. There are also small foci of mononuclear cells—lymphocytes, histiocytes and plasma cells. There is no marked bile duct proliferation.

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2. A second type of lesion which is quite common consists of greyish spherical or ovoid nodules measuring 1-5 mm. in diameter. These are most commonly situated just beneath the liver capsule, but are also found within the depth of the organ. They are often present together with lesions of the first type. In histological sections they consist of lymphoid tissue, surrounded by a fibrous capsule, from which trabeculæ pass inwards, dividing the nodule into small units which usually contain well-developed lymph follicles. Mitotic figures are numerous among the reticulum cells and lymphoblasts within the nodule. Nodules of this type were not found in any of the normal pig livers examined, and it is considered that they represent the formation of new lymphoid tissue as a reaction to parasitic migration. It is well known that lymphoid tissue can be formed from undifferentiated mesenchyme cells in connective tissue in various situations, including the periportal connective tissue in the liver. In one lymphoid nodule an immature fluke was found, whilst in another there was an ovoid structure containing calcium which resembled a degenerated nematode. In the liver of a pig affected with swine erysipelas, and also with "white spot liver," several lymphoid nodules were found in which there were large numbers of swollen reticular cells as well as giant cells, within the cytoplasm of which were large numbers of gram positive bacteria. It would seem, therefore, that such adventitious lymphoid nodules are able to play a part in phagocytosis of bacteria.

According to Dobberstein (1936), microscopic lymphoid nodules occur in the interlobular connective tissue of normal pig livers, and the same author, in dealing with parasitic nodules in the liver of the horse, states that the larvæ make their way to pre-existing lymphoid nodules where they undergo degeneration and produce lymphoid hyperplasia. These worm nodules in the horse were formerly believed to be due mainly to Strongyle larvæ, but it is now considered that ascaris larvæ are responsible for a considerable proportion.

3. A third type of lesion consists of spherical nodules from 3-8 mm. in diameter and usually superficial. They comprise an outer dense fibrous capsule and a central cavity containing a brownish or yellowish semi-solid material. Lesions of this type were received from the bacon factory previously referred to, and in eight such lesions from five different pigs parasites have been found which can be identified with certainty as flukes, probably young stages of *Fasciola hepatica*. Lesions of this type were not found in material from any of the other sources.

These parasites have been recorded in Germany from similar lesions in pig livers by Wolfhügel (1907) and by Bugge and Müller (1928). Bugge has published several articles on the development of *Fasciola hepatica* in domestic animals with especial reference to the morphological characters of immature flukes found in the lungs and mesenteric lymph nodes of cattle. In his most

recent article (Bugge, 1935), he states that the young cercariæ which reach the liver are unable to pass through the liver sinusoids because of their greater diameter. They possess backward projecting spines, and are well equipped for boring through the tissues, and they may occasionally penetrate the fœtus in utero. In the pig, especially, many flukes fail to reach the bile ducts and become encysted in the liver tissue, where they form abscesses up to the size of a hazel nut.

Description of Individual Cases.—The first five livers were from pigs of various ages from which small portions of tissue were received already fixed.

1. Pig, male, 46 days old, weight 6 lb. Killed. Enormous increase in eosinophils in the interlobular tissue and portal tracts and, to some extent, within the liver lobules. No foci of parenchyma destruction were seen. The intensity of the eosinophil infiltration can be judged from the fact that a cross-section of a single branch of the portal vein contained within its lumen over 200 eosinophils and a smaller accompanying artery contained 30.

2. Pig, female, 120 days old, weight 80 lb. Killed at abattoir. Condition similar to Pig 1 but less recent, there being considerable fibroblast proliferation and formation of collagen in the interlobular septa and portal tracts, the latter being infiltrated with eosinophils. One section showed a focus of necrosis and hæmorrhage involving the central two-thirds of a lobule and heavily infiltrated with eosinophils.

3. Pig, male, 30 days old, weight 6 lb. Undersized, destroyed. The interlobular septa were not yet clearly defined. In one section there was a small intralobular necrotic focus which, together with the neighbouring portal tracts, was heavily infiltrated with eosinophils. (Fig. 6.)

4. Pig, male, 140 days old, weight 70 lb. Killed at abattoir. All septa thickened by formation of fibrous tissue and infiltrated with large numbers of eosinophils. Some septa measured up to 800 microns in thickness as compared with the normal septa, which are about 10 microns. Very occasional neutrophils and lymphocytes were present.

5. Pig, female, 112 days old, weight 45 lb. Killed at abattoir. Septa were thickened as in the previous cases and contained several lymphocytic foci. One section showed a mass of inflammatory cells almost completely replacing two to three liver lobules and projecting slightly above the surface. The centre of this focus was occupied by a small ovoid pink structure containing minute blue dots and surrounded by a pink fibrillary material. This structure probably represents an oblique section of a parasite, and was embedded in a collection of lymphoid cells, outside which were enormous numbers of eosinophils. The parasite was 6-8 microns in width and the two portions seen in sections were 13 and 22 microns in length, respectively.

The next three cases were from small bacon pigs, portions of the livers of which were condemned.

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6-8. Eight lesions were sectioned. Six consisted of spherical nodules of lymphoid tissue situated near the surface and 1-1.5 mm. in diameter. They showed well-defined follicle formation and were surrounded by a fibrous capsule from which trabeculæ passed towards the centre of the nodule. Eosinophils were scanty within the nodules, but were present in considerable numbers in the capsule and neighbouring portal tracts. The remaining two lesions consisted of superficial wedge-shaped areas of dense fibrous tissue, the base of the wedge being situated just beneath the liver capsule and the apex extending along a portal tract. In the centre of each lesion was an aggregation of lymphoid cells without definite follicle formation, and in one of these was a pink staining mass similar to that surrounding the parasite in No. 5, but no definite evidence of a parasite was found.

Portions of liver were obtained from two small bacon pigs.

9-10. The two lesions sectioned consisted of lymphoid nodules with a capsule, follicles and connective tissue septa. There was no evidence of a parasite. Occasional eosinophils were found within the nodules, and in greater numbers in the surrounding septa.

Small portions of liver were obtained from 10 bacon pigs slaughtered at a large bacon factory.

11. Sections of seven lesions were examined. Two consisted of nodules 5 mm. in diameter with a central area of necrotic leucocytes, mainly eosinophils, and showing early calcification: this was surrounded by lymphoid tissue and by a thick fibrous capsule, the latter and the neighbouring septa being heavily infiltrated with eosinophils and thickened by newly formed fibrous tissue. The centre of both lesions showed a bluish ovoid structure, details of which were indistinct, but which from its appearance probably represented a degenerated parasite similar to the flukes found in other livers in this group of pigs.

12. Sections of six lesions were examined. One showed a superficial lymphoid nodule embedded in dense fibrous tissue and infiltrated with eosinophils. Four of the remaining lesions consisted of foci of necrosis and hæmorrhage in the central portion of single liver lobules. The liver capsule covering the superficial lesions was thickened by an intense eosinophil infiltration. The sixth lesion showed destruction of the central half of two superficial lobules and, in addition, a small focus of lymphoid cells surrounded by eosinophils. In the centre of the latter was a pink fibrillary material surrounding a parasite. (Fig. 10.) The parasite was found in eight sections and was estimated to be about 9 microns in diameter and about 200 microns in length. It consisted of a tubular structure comprising a pink staining cuticle and a central mass of bluish dots measuring about 1 micron. (Fig. 11.) The fibrillary material which enveloped it would seem to be either a product of the reaction of the tissues, perhaps related to fibrin, or a secretion of the parasite. The periphery

of this lesion showed fibrous tissue forming a capsule and extending along the neighbouring septa and portal tracts, all of which showed a marked eosinophil infiltration.

13. Three lesions were sectioned, two consisting of areas of dense fibrosis containing small lymphoid foci and infiltrated with eosinophils. The third lesion was a 2 mm. lymphoid nodule showing numerous follicles. Two sections through the centre of this lesion showed a small ovoid bluish structure, measuring 12 x 18 microns and probably representing a degenerated and calcified nematode. The parasite was surrounded by an eosinophilic fibrillary material similar to that already described.

14. Five lesions were sectioned. Three consisted of 3-5 mm. nodules composed of lymphoid tissue with a necrotic centre and an external collagenous capsule. The foci showed a central mass of coagulated material, stained intensely with eosin, in the midst of which was the outline of a degenerated parasite which measured in the three different lesions 1,200 x 660, 840 x 300 and 480 x 120 microns, respectively. The parasites showed a pink hyalin cuticle and an internal structure consisting of bluish reticular tissue. (Fig. 16.) Owing to the degenerated condition of the parasites, no further details of their structure could be made out. The parasites themselves and the portal connective tissue surrounding the lesions were infiltrated with eosinophils, and these cells formed thick "cuffs" around the vessels in the portal tracts and were present in large numbers within their lumen. The remaining two lesions were similar to the above, but showed central calcification without evidence of parasites.

15. The single lesion sectioned consisted of a superficial 1.5 mm. lymphoid nodule containing follicles and infiltrated with eosinophils. In the centre of some sections was seen a parasite in which the details of internal structure were well preserved. (Fig. 15.) It had an outer hyaline eosinophilic cuticle and its internal structure included one large and two small tubes cut in cross-section. The probable dimensions of the parasite, estimated from the sections available, were about 1,000 x 400 x 350 microns. Near the parasite were several groups of large pale-staining endothelioid-type cells, some of which were fused to form large syncytial masses.

16. Of the two lesions sectioned, one consisted of a 5 mm. focus with a centre of necrotic eosinophils surrounded by endothelioid-type cells, then by lymphoid tissue, and finally enveloped by a thick fibrous capsule. Some sections showed the remains of a degenerated parasite similar to those seen in No. 14. Near the parasite the endothelioid cells had formed syncytial masses. The other lesion was 1.8 microns in diameter, and consisted of hæmorrhage and necrosis with very little reaction other than a most intense eosinophil infiltration within it and for some distance along neighbouring portal tracts.

17. Two lesions were sectioned. One showed merely an increase in

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interlobular connective tissue, heavily infiltrated with eosinophils, the overlying liver capsule being thickened. The other lesion consisted of two small hæmorrhagic foci containing large numbers of eosinophils.

18. Four lesions were sectioned. One consisted of a 2-3 mm. hæmorrhagic focus which had destroyed the greater part of two superficial lobules. Two further lesions showed 1-1.5 mm. lymphoid foci surrounded by fibrous tissue and showing focal collections of eosinophils in the nearby connective tissue. The fourth lesion was a 1 mm. spherical lymphoid nodule projecting beyond the normal liver surface and showing well-defined follicles. Its centre showed in one section the remains of a calcified parasite measuring 11 x 30 microns and containing small bluish dots. Occasional eosinophils were present within the nodules and were more numerous in its capsule.

19. Seven lesions were sectioned. One lesion consisted of a 3-4 mm. lymphoid nodule surrounded by a fibrous tissue capsule and containing a central area of necrotic leucocytes, within which could be seen the shadowy outline of an ovoid parasite. The latter varied in size in different sections from 300 x 250 to 530 x 400 microns. No internal structure was visible and the parasite and surrounding necrotic tissue was undergoing early calcification. Two further lesions were similar to the above but rather smaller, and showed no evidence of parasites. The fourth lesion was the largest we have encountered, measuring 12-13 mm. and having a central 8 mm. cavity. The latter contained masses of degenerated eosinophils, a pink coagulum and a parasite which was cut in seventeen different sections, and was well preserved as regards its internal structure. (Fig. 17.) It was in the form of an elongated ovoid structure measuring approximately 900 x 400 x 100 microns. One section showed a depression at one end of the parasite suggestive of a sucker. (Fig. 17.) The three remaining lesions were in the form of 2-4 mm. lymphoid foci with a central mass of eosinophils surrounded by endothelioid cells, many of which had formed syncytial masses. (Fig. 7.) There were no parasites in these lesions.

20. Seven lesions were sectioned and all consisted of 1-2 mm. hæmorrhagic foci, intensely infiltrated with eosinophils, this infiltration extending along the neighbouring septa. (Fig. 14.)

A small portion of liver from each of two small bacon pigs was received fresh from the local abattoir.

21. The convex (anterior) surface showed four lesions, one of which consisted of a small central cyst-like structure surrounded by thickened septa. This lesion was incised and the contents examined in saline. A nematode parasite which is almost certainly an ascaris larva was found, and is shown in the drawing. (Fig. 19.)

The larva, which had unfortunately been ruptured, was first examined in the fresh state for morphological detail and a camera lucida drawing made

of it. Later it was fixed with as little disturbance as possible by running formol saline beneath the cover slip and the preparation was sealed and re-examined. The details of the mouth could not be accurately determined, but it appeared to be surrounded by three shallow lips. The œsophagus was somewhat more distinct in the fixed than in the unfixed state and appeared to be club-shaped with a tendency to form a bulb at the posterior end. The outline of the intestine was very difficult to determine as the parasite was packed with refractile granules, which were much denser in the intestinal region than in the œsophageal and caudal portions. Both excretory pore and anus were visible and the larva ended in a conical tail. No papillæ could be demonstrated on the lips of the head end, nor was the genital primordium visible in the living or fixed state. The principal measurements were as follows: length of larva, 233μ; maximum width 16μ; length of œsophagus 80μ; length of tail, measured from anal opening, 37μ; excretory pore 38μ from the head end. As the specimen was ruptured it was difficult accurately to determine its total length, and the measurement of 233μ is therefore open to question. From such morphological details as could be observed and from the measurements given above it would appear that the specimen was probably a second-stage larva of *Ascaris lumbricoides*. Roberts (1934) gives a description of a specimen of an *A. lumbricoides* second-stage larva, which is similar to that of the present material; his measurements were: length 244μ; œsophagus 89μ; excretory pore 45μ; anus 42μ.

The opened lesion was subsequently sectioned and consisted of a wedge-shaped mass of fibrous tissue containing a central cavity, the inner wall of which was formed by lymphoid cells. Eosinophils were very abundant in the thickened interlobular septa. (Fig. 13.)

22. Three small circumscribed whitish foci were present. One was a small lymphoid nodule seen in sections to consist of about 20 spherical follicles up to 250 microns in diameter. (Fig. 9.) Eeosinophils were fairly numerous in the capsule and surrounding septa and in the lumen of branches of the portal vein. A second lesion consisted of a wedge-shaped area of fibrous tissue heavily infiltrated with eosinophils and replacing 2-3 liver lobules. The third lesion showed an area of hæmorrhage and necrosis extending from the liver capsule to a depth of 1.5-2 mm. Two sections from the centre of this lesion showed a parasite measuring 7.5 x 12.5 microns, surrounded by eosinophils and degenerating liver cells. (Fig. 12.) No obvious fibroblast proliferation had yet occurred. The liver capsule over the lesion was thickened and infiltrated with eosinophils.

23. The liver of a bacon pig from the local abattoir condemned for "white spot liver" showed about 30 whitish lesions on each surface, most of them being solid nodules 1-3 mm. in diameter, from which radiated thickened interlobular septa. There were many similar lesions in the depth of the liver.

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Twelve lesions were examined in the fresh state, but no parasites were found. Nine lesions were sectioned. Seven consisted of 0.5-3 mm. lymphoid foci, most of which showed follicle formation and eosinophil infiltration, the latter being most marked in the capsule and surrounding portal tracts. Several of the nodules showed " foreign body " giant cells and occasional small aggregations of eosinophils, some of which were degenerating. The two remaining lesions differed in consisting of a large mass of fibrous tissue with a small central area of lymphoid tissue containing small collections of necrotic eosinophils. No parasites were seen in any of the nine lesions.

24. The liver of a second bacon pig from the same batch as No. 23 was examined and photographed. (Figs. 1-4.) The lesions were similar and twenty of them were examined in the fresh state. The only evidence of a parasite consisted of a number of tape-worm hooks found in a small spherical cyst which was quite unlike the other lesions. The hooks were examined by Dr. J. N. Oldham and from their measurements they probably represent a *Cysticercus tenuicollis*, the intermediate stage of *Tænia hydatigena*.

	Large Hooks.	*Small Hooks.*
Tænia hydatigena ...	0.17 —0.22 mm.	... 0.11 —0.16 mm.
Tænia solium ...	0.16 —0.18 mm.	... 0.11 —0.14 mm.
Pig No. 24	0.186—0.192 mm.	... 0.120—0.128 mm.

It was possible to divide the superficial lesions roughly into three types:

(a) Lesions consisting merely of thickened interlobular septa without the formation of a solid central focus. Sections of these showed grossly thickened septa up to 350 microns in width and heavily infiltrated with eosinophils. (Fig. 5.)

(b) Foci with a solid whitish centre and radiating thickened septa. These were similar to (a) but showed in their centre a lymphoid nodule about 1 mm. in diameter with a central degenerating mass of eosinophils.

(c) Spherical whitish nodules with no gross thickening of adjacent septa. These consisted of lymphoid tissue, surrounded by a fibrous tissue capsule infiltrated with eosinophils and with very little thickening of nearby septa.

The lesions situated in the depth of the liver were similar to (c) except for one very recent lesion consisting of a focus of hæmorrhage in which fibrosis had not yet occurred.

25. A small portion of the liver of a bacon pig with swine erysipelas was received from the local abattoir. It was swollen, congested, and the lobular pattern was very pronounced. There were several lesions similar to those described under No. 24. Sections showed central lobular necrosis and hæmorrhage. One focal lesion consisted of thickened interlobular septa infiltrated with eosinophils and containing a central area of lymphoid tissue. The latter showed a number of swollen reticulum cells as well as giant cells,

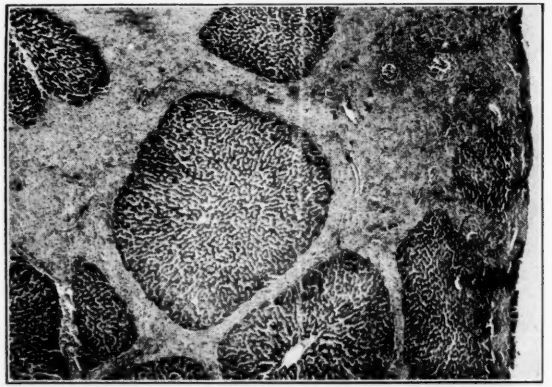

FIG. 5.—Superficial lesion from pig 24, consisting of fibrous thickening of interlobular septa, infiltrated with eosinophils. The septa at the extreme lower edge of the field are almost normal. Near the surface of the lesion are one or two small lymphoid foci. Hæmatoxylin and eosin. x 27.

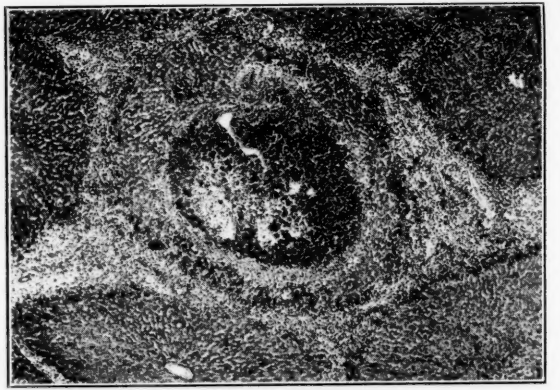

FIG. 6.—Early hæmorrhagic lesion from pig 3, showing hæmorrhage and necrosis involving the centre of a liver lobule. The lesion and the neighbouring septa show an intense eosinophil infiltration. Hæmatoxylin and eosin. x 33.

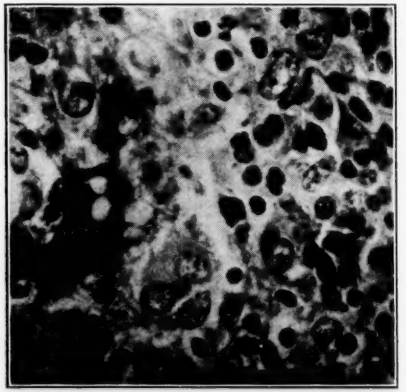

FIG. 7.—Portion of large lesion from pig 19, showing cells of endotheloid type, lymphocytes and syncytial masses with pyknotic nuclei. Hæmatoxylin and eosin. x 576.

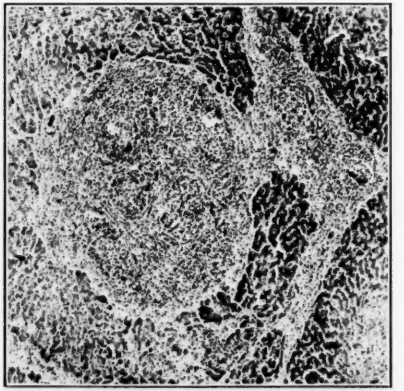

FIG. 8.—Lymphoid nodule within a liver nodule from pig 25. A tendency to follicle formation is seen and the portal tract to the right of the field shows two collections of lymphoid cells. Hæmatoxylin and eosin. x 54.

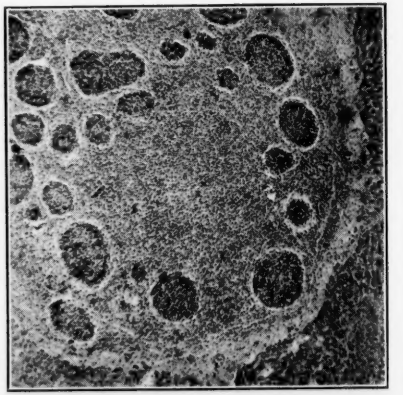

FIG. 9.—Lymphoid nodule from pig 22, showing follicle formation. A giant cell is seen to the right of the centre of the field, and the nodule is surrounded by a connective tissue capsule, infiltrated with a few eosinophils. Hæmatoxylin and eosin. x 45.

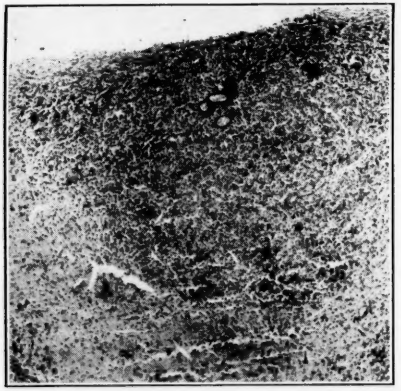

FIG. 10.—Superficial lesion from pig 12, showing nematode larva cut in three places and surrounded by a darkly staining fibrillary material. The liver capsule is absent over the centre of the lesion, and at either side shows a heavy eosinophil infiltration. Hæmatoxylin and eosin. x 54.

FIG. 11.—The parasite shown in Fig. 10 is illustrated under a higher magnification. The fibrillary material is clearly shown, as are the small circular dots within the parasite. The parasite is surrounded by mononuclear cells, there being few eosinophils in its immediate vicinity. Hæmatoxylin and eosin. x 288.

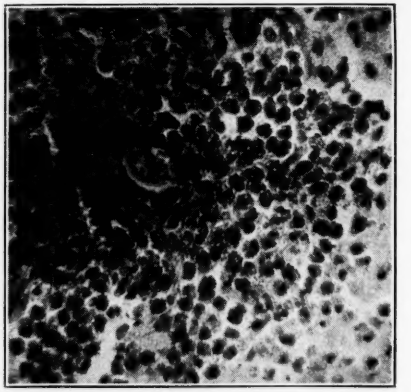

FIG. 12.—Section of nematode larva from pig 22, surrounded by a mass of eosinophils. Hæmatoxylin and eosin. x 540.

FIG. 13.—Opened lesion from pig 21, from which an ascaris larva was obtained by dissection in the fresh state. The central cavity is surrounded by lymphoid cells and embedded in a dense mass of fibrous tissue containing large numbers of eosinophils. Hæmatoxylin and eosin. x 27.

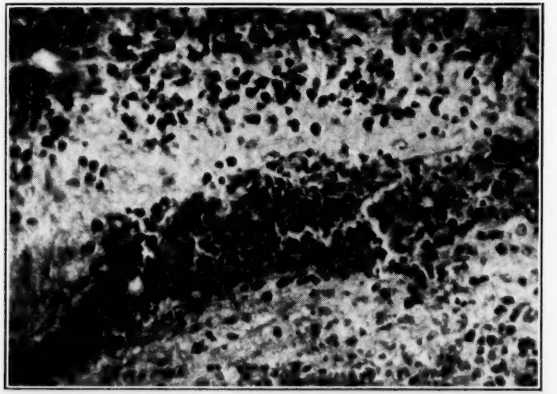

FIG. 14.—Section through a small branch of the portal vein in a lesion from pig 20, showing large numbers of eosinophils in the lumen of the vessel and infiltrating its wall and the surrounding portal connective tissue. Hæmatoxylin and eosin. x 234.

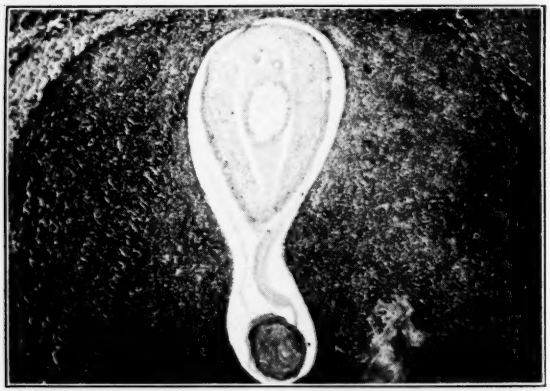

FIG. 15.—Lymphoid nodule from pig 15, showing a parasite, possibly an early stage of *Fasciola hepatica*. Hæmatoxylin and eosin. x 54.

FIG. 16.—Section through lesion from pig 14, showing a parasite, probably *Fasciola hepatica*, embedded in a central cavity filled with an exudate rich in eosinophils. The inner portion of the wall of the abscess is cellular and its outer portion consists of dense fibrous tissue showing eosinophil infiltration. A small portion of normal liver tissue is shown at the extreme upper edge of the field. Hæmatoxylin and eosin. x 33.

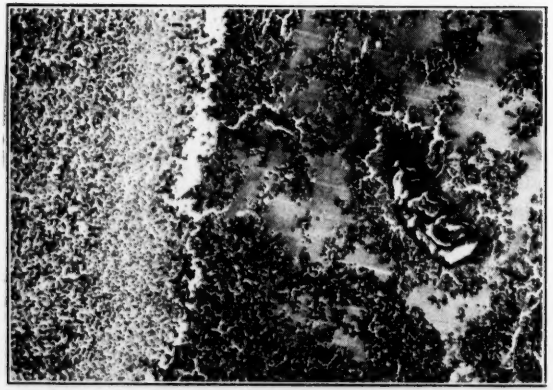

FIG. 17.—Section through an abscess from pig 19, showing a parasite, probably *Fasciola hepatica*, with a structure at its lower end suggestive of a sucker. The parasite is surrounded by a palisade of "clubs" and is embedded in a coagulated exudate very rich in eosinophils. The inner cellular zone of the abscess wall is shown to the left of the field. Hæmatoxylin and eosin. x 67.

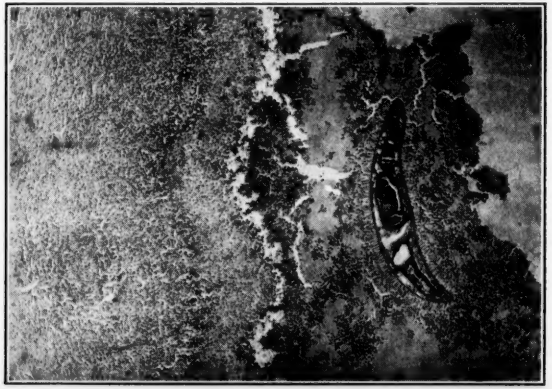

FIG. 18.—Same lesion as Fig. 17, showing the parasite cut through a different plane. The inner cellular and the outer fibrous portions of the abscess wall occupy the left half of the field. Hæmatoxylin and eosin. x 45.

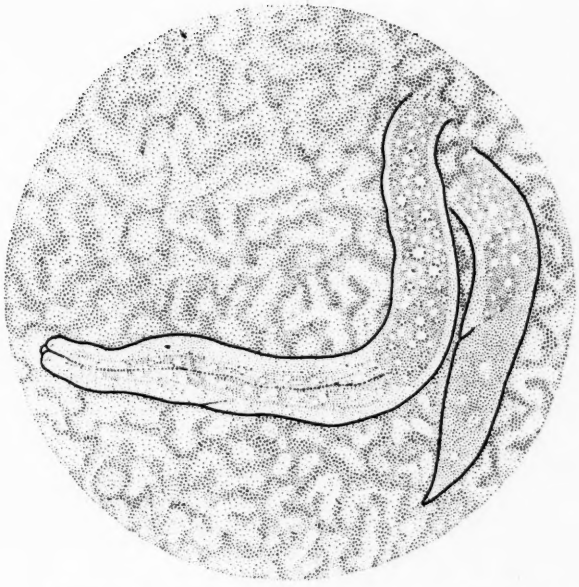

FIG. 19.—Camera lucida drawing of nematode larva isolated from Pig 21. x 665.

within the cytoplasm of which were masses of gram positive bacteria. Another lesion consisting of lymphoid tissue was situated within a liver lobule. (Fig. 8.) All the lesions were infiltrated with eosinophils, and these cells were present in large numbers in the adjacent septa. One lymphoid nodule showed a very well-developed follicular pattern, and in its centre was a small purplish structure which possibly represented a calcified nematode.

Small portions of liver from six bacon pigs were received from Edinburgh. The livers were condemned for lesions of " white spot liver."

26. The lesions consisted of hæmorrhagic foci with central lobular necrosis and intense eosinophil infiltration.

27 and 28. The lesions were similar to No. 26 but much more extensive. The septa were thickened and heavily infiltrated with eosinophils.

29. There was very extensive destruction of liver parenchyma and evidence of beginning fibroblast proliferation.

30. This showed a small lymphoid nodule containing follicles. The nearby septa were thickened and showed eosinophil infiltration.

31. This was similar to No. 29.

Discussion

Of the thirty-one livers examined, twenty-six showed lesions of the "white spot liver " type. Five of these (Nos. 5, 12, 18, 21 and 22) contained nematode larvæ, and two others (Nos. 13 and 25) showed structures which probably represented calcified larvæ. The larva isolated in the fresh state from Pig 21 was a second-stage ascaris and, from the size and morphology of the other nematodes seen in histological sections, there is little doubt that they also represent ascaris larvæ. The parasites seen in sections are naturally smaller than in the fresh state, due to the shrinkage which occurs during fixation, dehydration and embedding in paraffin.

The first stage in the development of the lesions would appear to be parenchyma destruction following the arrival of second-stage ascaris larvæ. The majority of larvæ are presumed to reach the liver by the portal blood, but some may possibly penetrate the liver capsule from the peritoneal cavity. Evidence in favour of the latter route was provided by the finding of thickening and eosinophil infiltration of the liver capsule covering several superficial lesions. Most of the ascaris larvæ probably complete their migration to the lungs, only an occasional parasite being held up in the liver and undergoing degeneration. One or several liver lobules, mainly their central portions, show hæmorrhage, degeneration and necrosis, and a very intense infiltration with eosinophils. This infiltration involves also the neighbouring interlobular septa, and large numbers of these cells can be seen within branches of the portal vein and hepatic artery, migrating through the vessel walls and arranged in the form of a " cuff " around the vessels. Fibroblast proliferation occurs in the

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septa and there is extensive formation of collagen fibres. The interlobular septa become greatly thickened, and may eventually be as much as eighty times their normal thickness. The parenchyma destruction is probably rapidly repaired, the damaged tissue being absorbed and replaced by newly formed liver cells, a process which occurs very rapidly in central lobular destruction produced in pigs by carbon tetrachloride administration (White, 1939b). In such cases septal thickening and eosinophil infiltration indicate the site of the original migration. It appears that in some lesions the area becomes occupied by a nodule of lymphoid tissue, often showing well-developed follicles. The connective tissue capsule surrounding these nodules shows eosinophil infiltration, as do the surrounding thickened septa. The combination of thickened septa and lymphoid foci accounts for the different types of lesion seen in "white spot liver." The number of eosinophils tends to diminish in older lesions, but this infiltration remains a characteristic feature.

If this view of the ætiology of "white spot liver" is correct, there is clearly a case for further measures of control of ascaris infestation in pigs in this country. The very considerable condemnation of livers is alone a serious loss, but a more important factor is the interference with the animal's health due to the pneumonic lesions in young pigs caused by ascaris larvæ, and the effects in older animals of the adult parasites in the small intestine. The widespread application of a swine sanitation system such as that recommended by the United States Federal Bureau of Animal Industry would do much to reduce the infestation and its ill effects. This system is adapted primarily to the control of the pig ascaris, but the basic principles apply also to the control of most of the important parasites of pigs. Full details of the system are given in the United States Department of Agriculture Farmers' Bulletin No. 1,787, "Internal Parasites of Swine," and in a special booklet, "The Prevention of Roundworm in Pigs," United States Department of Agriculture, Leaflet No. 5.

The larger and less common lesions found in five livers from a bacon factory contained immature flukes, and are similar to lesions already described in Germany. The failure of the parasite to reach the bile ducts is followed by its death and destruction within the liver, and there results an abscess, the wall of which is infiltrated with eosinophils and contains areas of lymphoid tissue. The relative rarity of adult flukes in the gall bladder and bile ducts of pigs in this country—they are occasionally found in sows—is probably due to the fact that a large proportion fail to complete their migration in this host.

Summary and Conclusions

The incidence of chronic focal interstitial hepatitis ("white spot liver") in pigs in Great Britain and Ireland has been investigated by means of a questionnaire, and the views of meat inspectors as to the condition have been

ascertained. The average incidence, based on the slaughter of over one and a half million pigs annually, was approximately 10 per cent.

As a result of the examination of twenty-six naturally occurring cases, it is concluded that the condition is parasitic in origin. This view is supported by a review of the literature dealing with similar conditions in other countries. From one typical lesion a second-stage ascaris larva was isolated. In addition, evidence of nematodes of comparable size and morphology was found in six other lesions among a total of seventy-five which were examined. The view is expressed that the common form of "white spot liver" is due to migrating ascaris larvæ. Most of the larvæ probably reach the lungs, and their passage through the liver results in the production of lesions which vary in character and extent according to the amount of parenchyma destruction and the interval after migration at which the liver is examined. Occasional parasites are held up in the liver and degenerate. Examination of large numbers of lesions at an early stage of their development may reveal the presence of migrating larvæ. The macroscopic and histological features of the lesions are described and illustrated.

From eight of fifteen considerably larger lesions evidence was obtained in histological sections of flukes which were probably immature examples of *Fasciola hepatica*. In addition, a spherical cyst in one liver contained a number of tapeworm hooks which, from their dimensions, were identified with a fair degree of certainty as *Cysticercus tenuicollis*, the intermediate stage of *Tænia hydatigena*.

In view of the high incidence of "white spot liver" and the considerable loss caused by the condemnation, partial or total, of affected livers, it is suggested that further measures should be adopted for the control of ascaris infestation. This is also of importance in view of the high incidence of pneumonic lesions in young pigs due to migrating ascaris larvæ and to the effect of the adult parasites in the small intestine.

Acknowledgments

I wish to thank my colleague, Dr. J. N. Oldham, for his help in identifying the parasites, and for preparing Fig. 19. I am also indebted to all those veterinarians and others who kindly supplied me with material and information, and to Mr. Macleod for preparing the photographs.

REFERENCES

Bugge, G. (1935): *Berl. tierärztl. Wschr., 51,* 65.
Bugge, G., and Müller, G. H. (1928): *Berl. tierärztl. Wschr., 44,* 293.
Dobberstein, J. (1936): *Ernst Joest's Handbuch der Speziellen pathologischen Anatomie der Haustiere,* Vol. 2 (first half), 2nd edition, Berlin.
Frenkel, H. S. (1920): *Z. Infekt Kr. Haustiere, 20,* 122.
Hall, M. C. (1924): quoted by Schwartz and Alicata (1933).

XUM

Joest, E., and Felber, W. (1908): *Z. InfektKr. Haustiere, 4,* 413.
Junack, M. (1907): *Z. Fleisch—u. Milchhyg., 17,* 164.
Kaupp, B. F. (1925): *Animal Parasites,* 4th Edition, London.
Mönnig, H. O. (1934): *Veterinary Helminthology and Entomology,* London.
Nieberle, K., and Cohrs, P. (1931): *Lehrbuch der Speziellen pathologischen Anatomie der Haustiere,* Jena.
Ostertag, R. von (1934): *Textbook of Meat Inspection* (English edition), London.
Roberts, F. H. S. (1938): Queensland—Animal Health Station, Yeerongpilly. Bulletin No. 1: "The Large Roundworm of Pigs."
Schmey, M. (1911): *Arch. wiss. u. prakt. Tierheilk., 37,* 591.
Schwartz, B., and Alicata, J. E. (1929): *J. Parasit., 16,* 168.
Schwartz, B., and Alicata, J. E. (1933): *J. Parasit., 19,* 17.
Schwartz, B., and Price, E. W. (1929): *Science 70,* 613.
Udall, D. H. (1939): *The Practice of Veterinary Medicine,* 3rd Edition, New York.
U.S. Department of Agriculture Farmers' Bulletin No. 1,787: "Internal Parasites of Swine." (Schwartz, B.)
White, E. G. (1939a): *J. Anat.,* London, *73,* 365.
White, E. G. (1939b): *J. Path. Bact., 49,* 95.
Wolfhügel, K. (1907): *Z. InfektKr. Haustiere, 2,* 546.

THE COMMONER CARRIERS OF SOUTH AFRICAN LARVAL TREMATODES

By F. GORDON CAWSTON
South Africa

UNLESS a fresh-water mollusc can readily be infested experimentally with the larva of an adult fluke it cannot be regarded as the usual intermediate host but, where a large proportion of shells are found to harbour typical larvæ in any one locality, it is reasonable to suppose that there is a correspondingly heavy infection of the final host.

Physopsis africana Krauss and its allied species which Morelet described as *Physopsis globosa* is remarkably likely to harbour one of the commoner schistosomes of man or stock wherever it is encountered throughout Africa, and it extends from Sierra Leone to Tanganyika and as far South as Humansdorp in the Cape Province.

Lymnæa natalensis Krauss possesses a more variable shell. It is one of the most common species in Southern Africa, and is sometimes infested with Fasciola. The shell is smaller and wider than that of *Lymnæa stagnalis* (Linnæus) and considerably larger than that of *Lymnæa truncatula* Müller, which is believed to be the more common intermediate host.

Planorbis pfeifferi Krauss, now to be known as *Biomphalaria pfeifferi* (Krauss), has a flattened shell and is distributed all over the Union of South

The commoner carriers of South African larval trematodes.

Africa and Central Africa, where it attaches itself to plants in shallow water and occasionally harbours schistosomes, though more commonly it harbours distomes.

Some of these cercariæ from *Biomphalaria pfeifferi* (Krauss) have a characteristic movement resembling that of skates and rays, their body and tail being similarly shaped, unlike that of furcocercariæ, which are more active and swim backwards. Very few of these larvæ have been determined by experimental means, but details of their structure have been recorded.

Only confusion is likely to arise by attempting to give distinctive names to all the varieties of mollusc found in pools from which cattle drink and vleis on which they graze, and a large number in South Africa may well be included under the species *Bulinus tropicus* (Krauss), which species is a common host of *Paramphistomum*.

Though *Melanoides tuberculata* Müller has been shown to serve as one of the two intermediate hosts required for the completion of the life-cycle of a trematode parasite of man in the Far East, experimental proof would appear to be lacking in support of the supposed identity of any of the cercariæ which infest this common shell of the African coast.

Though specially suited to withstand dessication in coastal areas, the mollusc is not uncommon in Pretoria, and this was the only species which survived more than a week or two when examples of *Lymnæa truncatula* Krauss, *Bulinus forskalii* Ehrenb. and others were sent me from that locality, its shell being operculated and too stout to be favoured as food for ducks.

A NOTE ON ACCURACY OF MEASUREMENT OF THE SKIN FOLD IN TUBERCULIN TESTS

By J. R. RIDER
Darlington

ASSUMING the use of an instrument of precision, it has long been the writer's view that accurate measurement of the skin fold is possible, and that it is a practicable procedure. The statement has been made "... it is possible to repeat measurements with an error of less than 0.5 m.m. Moreover, different observers can obtain readings within the same degree of accuracy." (Rider, 1940.)

An opportunity has occurred to verify this statement. Three observers, all having a considerable experience in the interpretation of the test, made

measurements of the skin folds on eight cows, each of which had been injected simultaneously with three tuberculins. The instrument used was a standard micrometer screw gauge reading to 0.01 m.m. Observer A was using the instrument for the first time, B had a longer experience with it, and C had used the gauge for many years. The following results were obtained.

The three observers agreed that each of the cows was negative to each of the three tuberculins.

THREE TUBERCULINS

Observers	*Variation* 0.5 *m.m. or less*	*Variation greater than* 0.5 *m.m.*	*Total*
A and B	20	4	24
A and C	18	6	24
B and C	21	3	24
	59 (81.94%)	13 (18.06%)	72

P.P.D. TUBERCULINS (MAMMALIAN AND AVIAN)

A and B	15	1	16
A and C	13	3	16
B and C	14	2	16
	42 (87.50%)	6 (12.50%)	48

WEYBRIDGE TUBERCULIN

A and B	5	3	8
A and C	5	3	8
B and C	7	1	8
	17 (70.83%)	7 (29.17%)	24

RANGE OF VARIATION

Observers	*Three Tuberculins*	*P.P.D.*	*Weybridge*
A and B	0.1—1.3	0.0—0.9	0.1—1.3
A and C	0.0—1.2	0.0—0.8	0.0—1.2
B and C	0.0—1.0	0.0—0.7	0.0—1.0

OCCURRENCE OF THE DIFFERENT VARIATIONS

	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	Total
Three Tuberculins ..	9	12	9	7	14	8	3	4	1	2	1		1	1	72
P.P.D. ..	5	9	7	5	9	7	2	2	1	1					48
Weybridge ..	4	3	2	2	5	1	1	2		1	1		1	1	24
	18	24	18	14	28	16	6	8	2	4	2		2	2	144

These results show that:

(a) Three observers were able to measure the same skin folds with a variation between them not greater than 0.5 m.m. in 82 per cent. of cases.

(b) The actual variation occurring most frequently was 0.4 m.m.

(c) Closer agreement was obtained with P.P.D. tuberculins than with Weybridge. This may be due to the fact that Weybridge tuberculin gave slightly larger and softer swellings, hence there was a greater possibility that the readings might be influenced by variations in pressure between the three observers.

These observations, made in the field, by workers with varying degrees of familiarity in the use of the instrument, on unselected cattle, support the writer's view that measurements can be repeated with a variation not exceeding 0.5 m.m., and that different observers can obtain the same degree of accuracy.

If, at some time in the future, the increase of thickness in the skin fold should become the criterion in the interpretation of the tuberculin test, it is clear that accurate measurement is possible. It is also evident that an instru-

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ment of precision, the micrometer screw gauge or some modification of it, must be used.

REFERENCE

Rider, J. R. (1940): "The Relation between Skin Measurements and the Character of the Swelling in Eleven Hundred Double Intradermal Tuberculin Tests." *Vet. J.*, *4*, 96.

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